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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> <p>This award funded four extremely productive years by two primary research teams (headed by Cronin at UMBC, Baltimore, MD and Marshall at University of Queensland, Brisbane, AUS). Nicholas Roberts, of Bristol University, UK, was a named collaborator on the project. The team members worked in Baltimore, Brisbane, Bristol, MBL Woods Hole, Duke University Marine Laboratory, and Lizard Island Research Station on the Great Barrier Reef. Other collaborators included Roger Hanlon (MBL) and Viktor Gruev (Washington University, St Louis). During the term of the grant, the Cronin team published (or have in press) 30 papers, 17 primarily supported by AFOSR. Seven additional papers are currently in preparation for submission by the end of 2013, with others to follow. Members of the Cronin team were invited to numerous international meetings during the grant's term. Our work has been featured in the international press, on RadioLab, and in many respected scientific blogs. Funding has produced new, unexpected information about materials and processes used to create, manipulate, and sense polarized light. It has initiated the design and construction of new devices to measure biological properties and the invention of new devices based on biological principles.</p>					
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**FINAL REPORT**  
**AFOSR Award Number FA9550-09-1-0149**

**"Natural Models for Autonomous Control of Spatial Navigation,  
Sensing, and Guidance"**

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May 28, 2013

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## **Objectives** *(as stated in the original proposal)*

### **(1) To measure and explain structural properties and their diversity in natural materials that preferentially reflect or absorb linearly and circularly polarized light.**

Our research has revealed that certain animals use very unusual structures to control the reflection, transmission, or absorption of linearly or circularly polarized light from their surfaces or in their photoreceptors. These structures often have no man-made counterparts, and the optics underlying polarization absorption or reflection are poorly understood. In this project we will examine new types of natural polarizers and polarization-sensitive photoreceptors, characterizing their spectral, ultrastructural, and theoretical optical properties.

### **(2) To investigate the physiological processing of polarization information.**

One group of our focal animal models, stomatopod crustaceans, possesses an astonishingly broad range of visual information-sensing channels. There are 16 information input channels in all: 12 are for the analysis of color (one also associated with polarized light) plus at least two for detecting linearly polarized light and two for circularly polarized light. Understanding how information is tracked in this parallel input array will be the basis for guiding research into sensor fusion, stimulus analysis, and post-processing of sensory information. Efficient processing and resultant “command decisions” for the brain to interpret and act on are essential for these fast-living, combative animals. Using electrophysiological and neuroanatomical studies on the properties of neurons in the visual pathway, we expect to discover new forms of sensory filtering, integration, and sorting. We now understand the basics of these properties, but new work will examine temporal features and spatial aspects of sensory processing with the goal of uncovering approaches to hyperacute imaging of stimuli in both time and space.

### **(3) To examine the design and specializations of molecular receptors used for visible and ultraviolet polarization sensing.**

Recent molecular research on photopigments in our laboratories, as well as newly published information on the 3-dimensional molecular structure of polarization-sensitive visual pigments, will form the background for new studies on molecular specializations of visual pigments and associated molecules that are designed for enhanced polarization sensitivity in both the visible and ultraviolet spectral ranges. With at least five UV photoreceptor classes, stomatopods have a particular interest in this spectral region that we currently do not understand. By working out the molecular underpinnings of polarization sensing, we will gain insight concerning functional differences among materials which could have application for fabrication or design.

### **(4) To characterize and explain polarization optics and visual sampling of natural stimulus fields in model organisms.**

A general principle of neural sampling is that both pre-receptor and post-receptor mechanisms operate in series to enhance signal detection. Our objective here is to examine some of the prefiltering mechanisms used, both optically and behaviorally. The work is expected to involve a variety of animals to explore diverse systems of managing difficult environmental challenges. These systems include object detection, covert signalling, and navigation.

## **Status of Effort**

The research funded by this award has led to four years of hard work by the two primary research groups (Cronin and Marshall), as well as for their students and collaborators. Our funded collaborator, Nicholas Roberts, also was an extremely active member of the research team. Cronin, Marshall, and Roberts joined forces to work in the field on polarization vision at the Lizard Island Research Station in Australia on two occasions, and met at several international meetings. All members of the team exchanged visits between their home laboratories on several occasions. We also invited a new collaborator, Viktor Gruev of Washington University (St Louis, MO), to join the team in the field for work on innovative polarization imaging systems. Research associated with this funding was carried out at UMBC, the University of Queensland, the University of California at Berkeley, Washington University, Bristol University, the Duke University Marine Laboratory, Lizard Island Research Station, and the Marine Biological Laboratory, Woods Hole.

During the term of the grant, we published (or have in press) 30 papers, of which 17 were primarily supported by Air Force funding. All members of the Cronin team presented numerous talks, seminars, and posters at international meetings, often with our major collaborators as coauthors. Our work has been featured in the international press, on RadioLab, and in numerous scientific blogs and online journals.

Funding has produced new, unexpected information about materials used by biological systems to produce, control, and analyze polarized light. We have made excellent progress towards understanding how polarized-light receptors in animals function as well, including their function at the molecular level. Funding by AFOSR has supported the modeling of complex biological reflectors, retarders, analyzers, and polarizers, some operating using previously undescribed optical mechanisms. It has also initiated the construction of new artificial devices to image and control polarized light, some of which will be used in our ongoing research, as well as by electrical engineers and computer scientists.

## **Accomplishments/New Findings**

1. Megan Porter, the postdoctoral fellow who was been funded by this award for the first three and a half years, has been a force of nature. She now has taken a permanent position at the University of South Dakota, and we miss her presence in the laboratory, although we continue numerous collaborations (and will work together in the field on AFOSR-related research this coming summer). Her 2012 paper in the *Proceedings of the Royal Society* continues as a highly-cited landmark contribution. In collaboration with Nick Roberts, she is preparing a manuscript on the properties of the opsins of deep-sea fishes, including their ability to withstand pressure, a significant study of how sensing proteins function in difficult environments, and the collaboration illustrates the strength of the scientific team that AFOSR has built. Her work on other aspects of the molecular design of visual (and nonvisual) opsins has opened new research territory now being pursued by her replacement (although she can never really be replaced), Hiroko Awata. For the entire term of the grant, Megan has been an essential authority for all work on the molecular genetics and molecular

biology of visual pigments, directing a number of independent projects. Megan presented a talk at the 2011 “Rank Prize Funds” meetings, where she was awarded “Best presentation by a junior scientist”. She was first or second author on many papers funded by this award, and has been a popular invited speaker at meetings around the world.

2. Hiroko Awata joined the laboratory in May, 2012 as the new postdoc, when it became apparent that Megan would probably be leaving the laboratory. She has worked throughout her first year on opsin expression and diversity in stomatopod retinas. Since I have not had the opportunity in past years’ reports to introduce her work, I included a small appendix showing some of her results. She attended two international meetings in 2012.

3. Nicholas Roberts, now of the University of Bristol in the UK, continued his highly valued collaboration with us on the biology and physics of polarized-light reception throughout the term of funding. Nick visited the laboratory on several occasions and spent several weeks with the team in the field on two occasions, working on problems in polarized-light perception and the optics of polarization reflectors. Nick has involved several of his students in this work as well. He also continues to use the UMBC server cluster for his modeling, using internet connections. Nick organized the “Rank Prize Funds” meeting on photoreception that occurred in June, 2011. We have several papers in preparation regarding the polarization receptors of mantis shrimps. Much of our mutual work has been reported by Nick’s team at meetings.

4. Michael Bok, a PhD student in the laboratory, will complete his thesis this year on the properties of ultraviolet (UV) polarization receptors in mantis shrimps. Mike attended a number of international meetings and was awarded the Crustacean Society’s “Best Oral Presentation by a Student” prize at the large SICB meeting in January 2012. (A second PhD student in my lab, Kathryn Feller – see below, was awarded the “Best Poster” prize, so the Cronin lab swept the awards in 2012; in 2013 we received honorable mentions at the same meeting). Mike and I are also working on a review of stomatopod sensory biology to be published in a major crustacean encyclopedia later this year. His thesis research, supported by AFOSR, will appear in major journals over the next year. He will begin a postdoc in the fall in Dan-Eric Nilsson’s laboratory at the University of Lund (where several AFOSR-funded projects are in place).

5. Although our formal collaboration with Nader Engheta has concluded, we continued to maintain contact with him on problems related to the biology of polarized-light perception. More significantly, we have enlisted one of his former postdocs, Viktor Gruev (now at Washington University, St Louis, MO), to work with us on polarization imaging and display options. Viktor spent a week with Justin, Nick, and me in the field last August working on projects and questions of mutual interests. The project we initiated at that time will continue through the term of our current AFOSR grant.

6. Our work with the Justin Marshall laboratory at the University of Queensland continues, as it has for many years, to be unusually productive, successful, and satisfying. My former PhD student, Tsyr-Huei “Short” Chiou, completed his postdoc in Justin’s group (with AFOSR support) and has now taken a permanent job in Tainan, Taiwan. Justin and I have

just completed our work on the jointly authored book *Visual Ecology* (to be published next year by Princeton University Press). Justin is responsible in part for the chapter on “Polarization Vision”. Our other authors for this book are Eric Warrant and Sonke Johnsen.

7. We worked throughout the term of the grant with Roger Hanlon’s laboratory at MBL Woods Hole, on non-visual opsins of cephalopods and fishes. My PhD student, Alexandra Nahm-Kingston, is now funded separately (by ONR) for this project. Cephalopods use identical opsins in their retinas and in their skin tissues, and we know the retinal opsins are designed to form multimeric polarization-oriented complexes, so we have become very interested to learn how these opsins are deployed in the distributed skin sensors in these animals. We also continue to work on polarized-light communication in these animals.

8. Kate Feller, a doctoral student in my laboratory (not primarily supported by AFOSR), worked throughout the project on the related topic of eye development in mantis shrimps and other crustaceans. Her work will help elucidate the molecular diversity in the retinas of these animals and produce a more complete understanding than we now have of the development of polarization vision and of the role of polarization opsins in larval and adult eyes. Kate attended several meetings over the term of the grant and has written two major reviews and a reviewed paper.

## **Personnel Supported**

### **Principal Investigator:**

Thomas W. Cronin, Dept. Biol. Sci, UMBC (Summer salary 2009-2012)

### **Postdoc:**

Megan L. Porter (Full-time salary through July, 2012)

Hiroko Awata (Full-time salary after May, 2012)

### **Graduate Students:**

Michael Bok (UMBC PhD student, Research Assistant 2012 - 2013)

Kate Feller (UMBC PhD student, research supported 2010-2013)

Alexandra Nahm-Kingston (UMBC PhD student, research supported 2010-2011)

### **Undergraduate Students (Research projects related to the AFOSR grant):**

Margaret Kott (UMBC junior, 2011)

Lara Seidman (UMBC freshman, Meyerhoff scholar, 4.0 GPA student, 2012)

Shelly Lai (UMBC junior, Honors undergraduate, 4.0 GPA student, 2012-2013)

## **Publications (\* indicates AFOSR primary support; reprints available on request)**

1. \*M.L. Porter, D.I. Speiser, S. Zaharoff, R.L. Caldwell, T.W. Cronin, and T.H. Oakley. (in press) The evolution of complexity of in visual systems of stomatopods: insights from transcriptomics. Integrative and Comparative Biology.

2. \*N.W. Roberts, N.J. Marshall, and T.W. Cronin. 2012. High levels of reflectivity and pointillist structural color in fish, cephalopods, and beetles (letter). *Proceedings of the National Academy of Sciences of the USA* 109:E3387.
3. T.W. Cronin. 2012. Visual optics: Accommodation in a splash. *Curr. Biol.* 22:R871-R873.
4. T.M. Frank, S. Johnsen, and T.W. Cronin. 2012. Light and vision in the deep-sea benthos: II. Vision in deep-sea crustaceans. *Journal of Experimental Biology* 215:3344-3353.
5. T.W. Cronin and K. Feller. (in press) Sensory ecology of vision. In: *Biology of Crustacea*, vol. 3 (C. Derby and M. Thiel, eds.) Academic Press, New York.
6. N. Bishoff, B. Nickle, T.W. Cronin, S. Velasquez, and J.I. Fasick. 2012. Deep-sea and pelagic rod visual pigments identified in mysticete whales. *Visual Neuroscience* (in press)
7. \*T.-H. Chiou, A.R. Place, R.L. Caldwell, N.J. Marshall, and T.W. Cronin. 2012. A novel function for a carotenoid: astaxanthin used as a polarizer for visual signalling in a mantis shrimp. *Journal of Experimental Biology* 215:584-589
8. \*M.L. Porter, J.R. Blasic, M.J. Bok, E.G. Cameron, T. Pringle, T.W. Cronin, and P.R. Robinson. 2012. Shedding new light on opsin evolution. *Proceedings of the Royal Society of London B* 279:3-14.
9. \*J. Marshall and T.W. Cronin. 2011. Polarization vision. *Current Biology* 21:R101-R105.
10. \*J. Marshall, T. W. Cronin, and Martin F. Wehling. 2011. Introduction: New directions in the detection of polarized light. *Philosophical Transactions of the Royal Society of London B* 366: 615-616.
11. \*T.W. Cronin and J. Marshall. 2011. Patterns and properties of polarized light in air and water. *Philosophical Transactions of the Royal Society of London B* 366:619-626.
12. \*N.W. Roberts, M.L. Porter, and T.W. Cronin 2011. The molecular basis of mechanisms underlying polarization vision. *Philosophical Transactions of the Royal Society of London B* 366: 627-637.
13. \*T.-H. Chiou, N.J. Marshall, R.L. Caldwell, and T.W. Cronin. 2011. Changes in light reflecting properties of signaling appendages alter mate choice behaviour in a stomatopod crustacean, *Haptosquilla trispinosa*. *Marine and Freshwater Physiology and Behaviour* 44:1-11.

14. J.H. Cohen, T.W. Cronin, N. Lessios, and R.B. Forward. 2010. Visual physiology underlying orientation and diel behavior in the sand beach amphipod *Talorchestia longicornis*. *Journal of Experimental Biology* 213: 3843-3851
15. \*M.L. Porter, Y. Zhang, S. Desai, R.L. Caldwell, and T.W. Cronin. 2010. Evolution of anatomical and physiological specialization in the compound eyes of stomatopod crustaceans. *Journal of Experimental Biology* 213: 3473-3496.
16. \*T.W. Cronin, M.L. Porter, M.J. Bok, J.B. Wolf, and P.R. Robinson. 2010. The molecular genetics and evolution of colour and polarization vision in stomatopod crustaceans. *Ophthalmic and Physiological Optics* 30: 460-469.
17. A.B. Lall, M.V.A. Järvilehto, T.W. Cronin, A.A. Carvalho, J.M. de Souza, M.P. Barros, C.V. Stevani, E.J.H. Bechara, D.F. Ventura, V.R. Viviani, and A.A. Hill. 2010. Vision in click beetles (Coleoptera: Elateridae): Pigments and spectral correspondence between visual sensitivity and species bioluminescence emission. *Journal of Comparative Physiology A* 196: 629-638.
18. B.E. Dalton, T.W. Cronin, N.J. Marshall, and K. Carleton. 2010. The fish eye view: Are cichlids conspicuous? *Journal of Experimental Biology* 213: 2243-2255.
19. \*T.W. Cronin. 2010. Polarized-light vision in land and aquatic animals. *Encyclopedia of the Eye*, vol. 3 (Darlene A. Dartt, editor). Oxford: Academic Press, pp. 461-468.
20. \*T.W. Cronin. 2009. Polarized-light vision in land and aquatic animals. *Encyclopedia of the Eye* (in press).
21. T.W. Cronin. 2009. Color vision in mantis shrimp. In: *McGraw-Hill Yearbook of Science & Technology*. McGraw-Hill Press, New York (in press).
22. C.M. Hofmann, K.E. O'Quin, N.J. Marshall, T.W. Cronin, O. Seehausen, and K.L. Carleton. 2009. The eyes have it: regulatory and structural changes both underlie cichlid visual pigment diversity. *PLoS Biology* 7:1-13.
23. \*N.W. Roberts, T.-H. Chiou, N.J. Marshall, and T.W. Cronin. 2009. A biological quarter-wave retarder with excellent achromaticity in the visible wavelength region. *Nature Photonics* 3, 641-644.
24. \*T.W. Cronin, T.-H. Chiou, R.L. Caldwell, N. Roberts, and J. Marshall. 2009. Polarization signals in mantis shrimps. *Proceedings of SPIE* 7461. Polarization Science and Remote Sensing IV (J.A. Shaw and J.S. Tyo, eds.). SPIE Press, Bellingham, WA. pp. 74610C-1 – 74610C-10.



25. L. Kiere, C.M. Hofmann, K. Omland, T.W. Cronin, and J. Price. 2009. Discrete evolutionary color changes in caciques suggest different modes of carotenoid evolution between closely related taxa. *Journal of Avian Biology* 40:605-613.
26. A.G. Cheroske, T.W. Cronin, M.K. Durham and R.L. Caldwell. 2009. Adaptive signalling behaviour in stomatopods under varying light conditions. *Marine & Freshwater Behaviour & Physiology* 42:219-232.
27. \*M.L. Porter, M. Bok, P.R. Robinson, and T.W. Cronin. 2009. Molecular diversity of visual pigments in Stomatopoda (Crustacea). *Visual Neuroscience* 26: 255-266.
28. A.B. Lall, T.W. Cronin, E.J.H. Bechara, C. Costa, and V.R. Viviani. 2009. Visual ecology of bioluminescent beetles: visual spectral mechanisms and the colors of optical signaling in Coleoptera, Elateroidea: Lampyridae, Elateridae, and Phengodidae. In: *Bioluminescence in Focus – A Series of Illuminating Essays* (V.B. Meyer-Rochow, ed.). Research Signpost Press, Kerala, India, pp. 201-228.
29. M.L. Porter and T.W. Cronin. 2009. A shrimp's eye view of evolution: how useful are visual characters in decapod phylogenies? *Crustacean Issues* 18: Decapod Crustacean Phylogenetics (J.W. Martin, K.A. Crandall, and D.L. Felder, eds.). CRC Press, Boca Raton, pp. 183-195.
30. \*M.L. Porter and T.W. Cronin. 2009. Evolution of mantis shrimp vision. *Kagaku* 79:660-666 (in Japanese).

**Note: Seven additional papers are in preparation from work funded by this grant, all for submission in 2013.**

## **Interactions/Transitions**

### *a. Participation/presentations at meetings, conferences, seminars, etc.*

#### Cronin – Invited academic seminars:

Georgetown University on October 22, 2009.  
 Duke University, January 25, 2010.  
 UMBC (Physics Dept.), February 17, 2010.  
 Purdue University, November 17, 2010.  
 UMBC, Department of Biological Sciences, February 23, 2011.  
 Duke University Marine Laboratory, February 25, 2011.  
 Graduate University for Advanced Studies, Sokendai, Hayama, Japan, July 27, 2011.

#### Cronin – Invited presentations at international meetings:

International Colour Vision Society, Braga, Portugal on July 27, 2009.  
 SPIE, San Diego, August 3, 2009.

“Light and Color in the Open Air” Meeting, St. Marys, MD, June 26, 2010.  
Jim Bowmaker’s retirement fest, London, England, June 25, 2011.  
“Rank Prize Funds” meeting on photoreception, Grasmere, England, June 30, 2011.  
5<sup>th</sup> Asia and Oceania Conference on Photobiology, July 30-August 1, 2011.  
AVA Meeting, University of Sussex, England, September 9, 2012.

Porter (postdoc) – Invited or contributed presentations at international meetings:

International Congress of Neuroethology, Salamanca Spain, August 2-7, 2010.  
FASEB, Biology and Chemistry of Vision, Carefree, Arizona, June 19, 2011.  
“Rank Prize Funds” meeting on photoreception, Grasmere, England, June 30, 2011.  
SICB, Charleston, SC, January 3-7, 2012.  
International Congress of Neuroethology, College Park, MD, August 6-10, 2012.  
AVA Meeting, University of Sussex, England, September 9-10, 2012.  
SICB, San Francisco, January 2-7, 2013.

Michael Bok (doctoral student) – Presentations at international meetings:

International Congress of Neuroethology, Salamanca Spain, August 2-7, 2010.  
“Rank Prize Funds” meeting on photoreception, Grasmere, England, June 30, 2011.  
SICB, Charleston, SC, January 3-7, 2012.  
International Congress of Neuroethology, College Park, MD, August 6-10, 2012.  
AVA Meeting, University of Sussex, England, September 9-10, 2012.  
SICB, San Francisco, January 2-7, 2013.

Kathryn Feller (doctoral student) – Presentations at international meetings:

International Congress of Neuroethology, Salamanca Spain, August 2-7, 2010.  
SICB, Charleston, SC, January 3-7, 2012.  
International Congress of Neuroethology, College Park, MD, August 6-10, 2012.  
AVA Meeting, University of Sussex, England, September 9-10, 2012.  
SICB, San Francisco, January 2-7, 2013.

Alexandra Nahm-Kingston (doctoral student) – Presentations at international meetings:

SICB, Charleston, SC, January 3-7, 2012.  
International Congress of Neuroethology, College Park, MD, August 6-10, 2012.  
AVA Meeting, University of Sussex, England, September 9-10, 2012.  
SICB, San Francisco, January 2-7, 2013.

\* Graduate students and undergraduates supported by this grant also presented numerous seminars, posters, and other presentations at UMBC throughout the term of funding.

*b. Consultative and advisory functions to other laboratories and agencies, especially Air Force and other DoD laboratories.*

We worked throughout the term of funding with Justin Marshall, University of Queensland, Australia, who is supported for joint projects by the AFOSR international office, Tokyo. Our collaborations took place in Baltimore, at various international meetings, at the University of

Queensland, and at the Lizard Island Research Station. We continue our work together on a current AFOSR grant.

We worked closely with Roy L. Caldwell, University of California Berkeley, sharing experimental animals. Dr. Caldwell also assisted us with critical field work in Australia.

We worked very closely throughout the term of funding with our major collaborator, Nicholas Roberts of Bristol University, England. Nick came to the US on several occasions and we visited him there. We also worked together at the Lizard Island Research Station.

We initiated a project with Viktor Gruev, Washington University, St. Louis. Viktor is now a supported subcontractor on our current AFOSR grant.

We continued collaborations with Roger Hanlon, Marine Biological Laboratory, Woods Hole, MA, to work on polarization sensing and reflections in cephalopod mollusks (squid, cuttlefish).

*c. Transitions.*

None as of yet, other than our new informal (now formal) collaboration with Viktor Gruev.

### **New Discoveries, Inventions, or Patent Disclosures**

None, other than those already mentioned and described in detail above.

### **Honors/Awards**

I was recognized as the UMBC “Presidential Research Professor” in April, 2009. I held this position for three years, from July 1, 2009 – June 30, 2012.

## ***APPENDIX: Work by Hiroko Awata, PhD***

Dr. Awata has been sequencing opsins from the retina of our target species of stomatopod crustacean, *Neogonodactylus oerstedii*. She has followed the sequencing with the creation of probes for *in situ* hybridization experiments in order to localize expression of the various opsin proteins. The major finding of her first year's work is that there is enormous co-expression of opsins in many classes of receptors. There is also some preliminary evidence that males and females of this species might differ subtly in the expression patterns of their opsins, including the types that contribute to polarized-light vision. This offers the possibility of sexually dimorphic polarization vision, which if true opens a host of questions about the fundamental biological functions and role of this sensory submodality.

Included in this appendix:

**Table 1:** Table of all labeling experiments completed to date, all from the mantis shrimp species *Neogonodactylus oerstedii*. The first column indicates the opsin number or clone number used to label, the name under this is the person who carried out the labeling experiment (Megan Porter to June, 2012; Hiroko Awata after that date). Sex is M (male), F (female), or ? (unknown).

“-” indicates no labeling; the number of “+” signs indicates the strength of labeling. DH – dorsal hemisphere of the compound eye. VH – ventral hemisphere. R – midband row (from 1 to 6). D – distal tier of row (for rows 1-4). P – proximal tier of row (for rows 1-4).

Note that the two rows devoted to polarization sensing (Rows 5 and 6), as well as the receptors of the dorsal and ventral hemisphere (which are also polarization-sensitive) express multiple visual pigments, usually with strong labeling. This has not ever been reported in any visual system previously.

A phylogeny of the relatedness of the genes used in *in situ* labeling is given below the final data set in the table.

**Figures 1 and 2** show samples of the actual *in situ* labeling data used to construct Table 1.

**Table 1: *In situ* labeling using probes developed against different opsin genes (see phylogeny below the table for gene relationships).**

No6=c12	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Megan	?	-	-	-	-	-	-	-	-	-	++	++	-
Hiroko	M	-	-	-	-	-	-	-	-	-	+++	+++	-
Hiroko	F	-	-	-	-	-	-	-	-	-	++	++	-
Hiroko	M	-	-	-	-	-	-	-	-	-	++	++	-
Hiroko	F	-	-	-	-	-	-	-	-	-	++	++	-

No7=c12	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Megan	?	-	-	-	-	-	-	-	-	-	++	++	-
Hiroko	M	++	-	-	-	-	-	-	-	-	++	++	++
Hiroko	F	+	-	-	-	-	-	-	-	-	++	++	+
Hiroko	M	-	-	-	-	-	-	-	-	-	+++	+++	-
Hiroko	F	+	-	-	-	-	-	-	-	-	+++	+++	+

No8=c13	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Megan	?	-	-	-	-	-	-	-	-	-	++	++	-
Hiroko	M	++	-	-	-	-	-	-	-	-	+++	+++	++
Hiroko	F	++	-	-	-	-	-	-	-	-	++	++	++
Hiroko	M	++	-	-	-	-	-	-	-	-	++	++	++
Hiroko	F	++	-	-	-	-	-	-	-	-	++	++	++
Hiroko	M	++	-	-	-	-	-	-	-	-	++	++	++
Hiroko	F	++	-	-	-	-	-	-	-	-	++	++	++

No12	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Hiroko	M	-	-	-	-	-	-	-	-	-	+++	+++	-
Hiroko		+	-	-	-	-	-	-	-	-	+++	+++	+
Hiroko	F	++	-	-	-	-	-	-	-	-	+++	+++	++
Hiroko	M	-	-	-	-	-	-	-	-	-	+++	+++	-
Hiroko	M	+	-	-	-	-	-	-	-	-	+++	+++	+
Hiroko	F	++	-	-	-	-	-	-	-	-	+++	+++	++
Hiroko	M	+	-	-	-	-	-	-	-	-	+++	+++	+
Hiroko	F	++	-	-	-	-	-	-	-	-	+++	+++	++

No9=GrpC	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Megan	?	-	++	++	++	++	++	++	++	++	-	-	-
Hiroko	F	+++	+	+	+++	+++	+++	+++	+	+	+++	+++	+++
Hiroko	M	++	-	-	-	-	-	-	-	-	+++	+++	++
Hiroko	M	+	++	++	++	++	++	++	++	++	+	+	+
Hiroko	F	+++	+	+	+	+++	+	+	+	+	+	+	+++
Hiroko	M	+++	+	+	+	+++	+	+	+	+	+	+	+++
Hiroko	F	+++	+	+	+	+++	+	+	+	+	+	+	+++
Hiroko	M	-	+	+	+	+	+	+	+	+	-	-	-
Hiroko	F	-	+	+	+	+	+	+	+	+	-	-	-

No13	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Hiroko	M	++	-	-	+	+	+++	+++	-	-	+	+	++
Hiroko	F	+++	-	-	++	++	+++	+++	-	-	++	++	+++
Hiroko	M	++	-	-	-	-	+++	+++	-	-	-	-	++
Hiroko	M	++	-	-	-	-	+++	+++	-	-	-	-	++
Hiroko	F	+++	-	-	++	++	+++	+++	-	-	++	++	+++
Hiroko	F	+++	-	-	++	++	+++	+++	-	-	++	++	+++

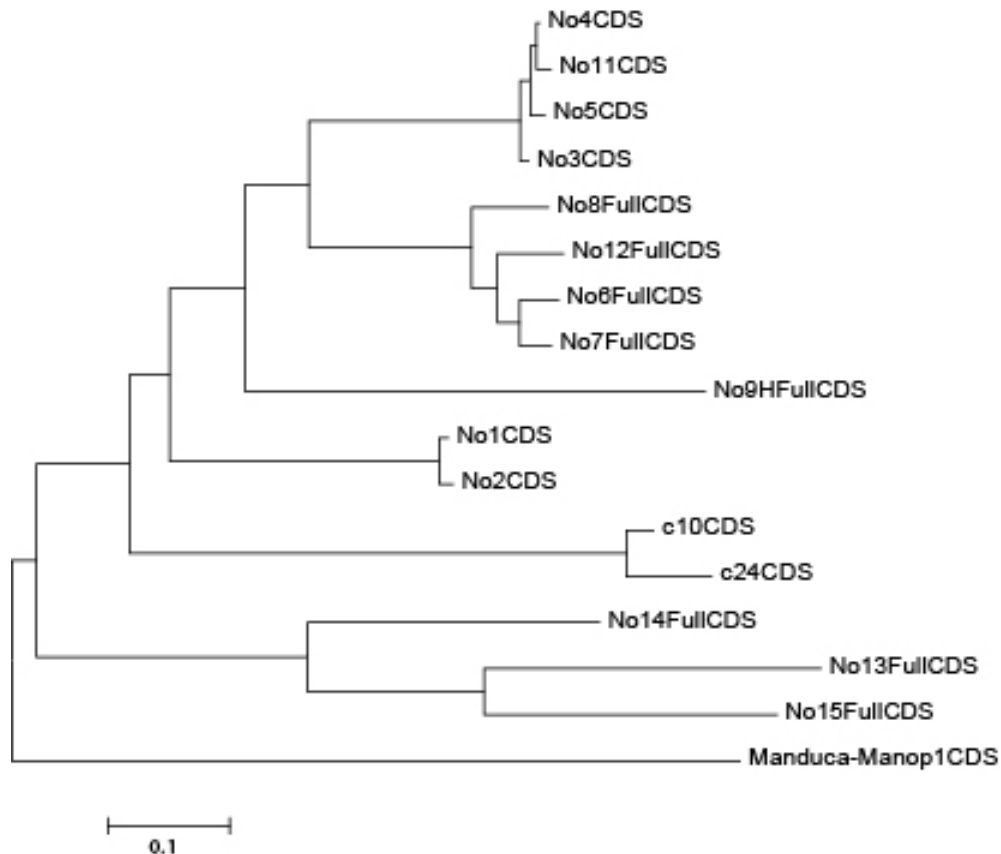
No14=c2	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Alastair	?	++	-	-	-	-	-	-	-	-	-	-	++
Megan	?	++	-	-	++	+	-	-	-	-	-	-	++
Megan	?	++	-	-	-	-	-	-	-	-	-	-	++
Hiroko	?	++	-	-	-	-	-	-	-	-	-	-	++
Hiroko	M	++	-	-	-	-	-	-	-	-	-	-	++
Hiroko	F	+	-	-	-	-	-	-	-	-	-	-	+

No15=c16	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Megan	?	++	-	-	-	-	-	-	-	-	-	-	++
Hiroko	M	+++	-	-	++	++	+++	+++	-	-	++	++	+++
Hiroko	F	+++	-	-	++	++	+++	+++	-	-	++	++	+++
Hiroko	M	+++	-	-	++	++	++	+++	-	-	++	++	+++
Hiroko	F	+++	-	-	++	++	++	+++	-	-	++	++	+++

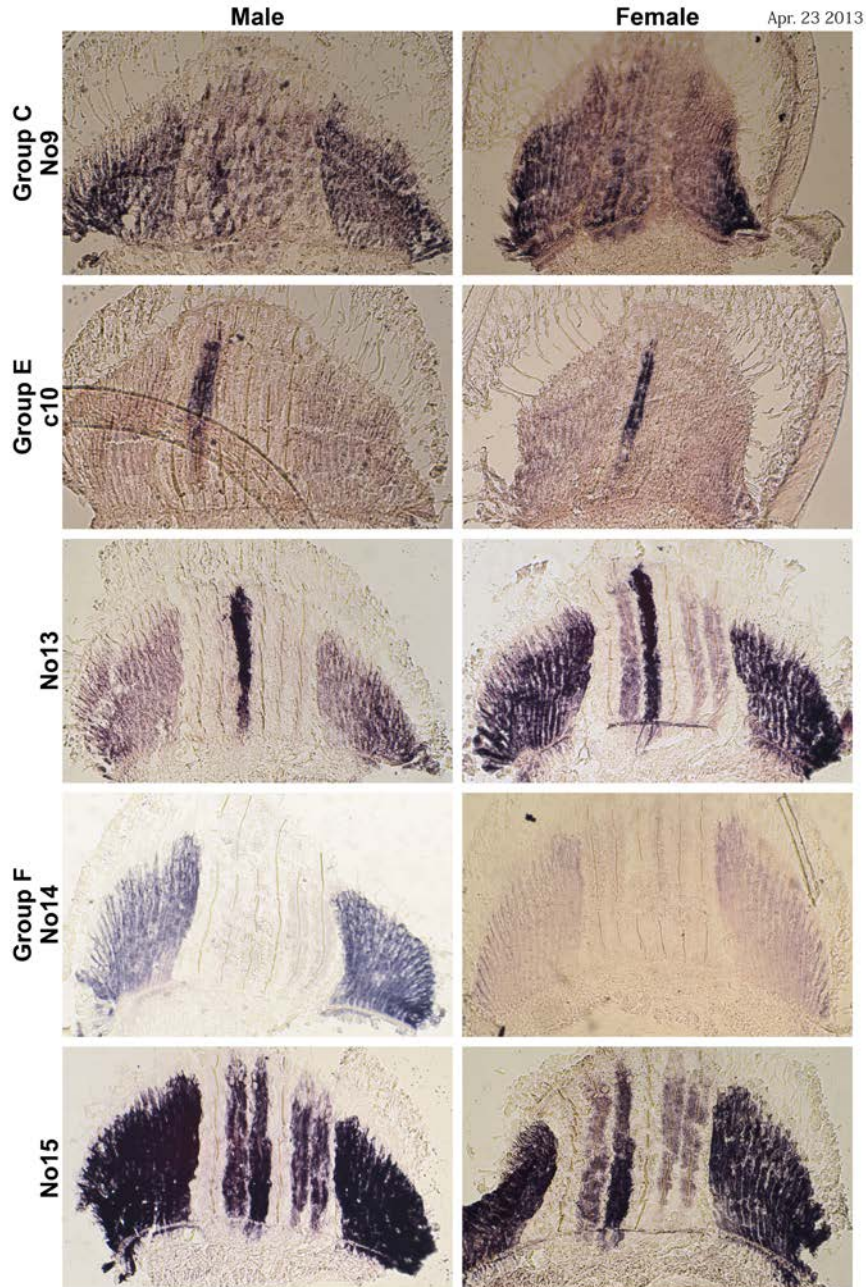
c10	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Megan	?	-	-	-	++	+	-	-	-	-	-	-	-
Hiroko	M	+	-	-	+++	+++	-	-	-	-	+	+	+
Hiroko	F	+	-	-	+++	+++	-	-	-	-	+	+	+
Hiroko	M	+	-	-	+++	+++	-	-	-	-	-	-	+
Hiroko	F	+	-	-	+++	+++	-	-	-	-	-	-	+

c24	Sex	DH	Mid band										VH
			R1D	R1P	R2D	R2P	R3D	R3P	R4D	R4P	R5	R6	
Hiroko	M	-	-	-	+++	-	-	-	-	-	-	-	-
Hiroko	F	-	-	-	+++	-	-	-	-	-	-	-	-

**Phylogeny showing evolutionary relationships among the opsin genes used to create the *in situ* probes.** CDS = coding sequence; Full CDS = entire coding sequence for the opsin gene. Note that opsins that are genetically far apart (on very different branches) can produce similar labeling patterns. The outgroup used to root the phylogeny is an opsin from the sphinx moth *Manduca sexta* (Manop1CDS).



**Figure 1. Sample in situ data; opsins No9, c10, No13, No14, and No15.** The dark blue or black color shows the label. The retina is sectioned in long section; the grouped ommatidia at the left and right are the Dorsal and Ventral hemispheres; Rows 1 to 6 are the vertical strips of cells. Most label is in the dorsal or ventral hemisphere and in ommatidia of Rows 2, 5, and 6. No13 most strongly labels Row 3; however, and No15 also labels Row 3 in addition to DH, VH, and Rows 2, 5, & 6.





**Figure 2. Sample in situ data; opsins No6, No7, No8, and No12.** The dark blue or black color shows the label. The retina is sectioned in long section; the grouped ommatidia at the left and right are the Dorsal and Ventral hemispheres; Rows 1 to 6 are the vertical strips of cells. Most label is in the dorsal or ventral hemisphere and in ommatidia of Rows 2, 5, and 6.

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